Biological Effects of Ingested Asbestos

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Biological Effects of Ingested Asbestos: Report and Commentary

by Douglas H. K. Lee*

Background

While there has been growing concern over the last twenty years, coupled with intensive research, about the pathogenic effects of inhaled asbestos, relatively little attention has been given to the biological effects of ingested asbestos. Research workers and public health officials have not been unaware of this gap, but the significance of the respiratory tract as a portal of entry was so obvious and demanded so much attention that there was some reluctance to divert any sizeable portion of resources or manpower to what was apparently the less pressing question.

Early in 1973 fairly extensive pollution of Lake Superior waters with asbestos from mine residues was recognized in the vicinity of the water intake for the city of Duluth, Minn. The subsequent furor over possible health consequences underscored the inadequacy of our knowledge about the implications of oral intake. From what we know of the physiology of the respiratory tract, it was assumed by many that the appearance of abdominal tumors following exposure to asbestos dust could be explained by swallowing of dust carried up the bronchial tree from deeper lung airways on the mucociliary "escalator". On the basis of this assumption it seemed only reasonable to suppose that the ingestion of asbestos fibers in water or food would increase the risk of developing similar abdominal tumors. But, it had to be admitted, direct proof of this conclusion was tenuous.

As an essential step in consolidating our knowledge about the risks involved, and in planning future inquiries to extend that knowledge, the National Institute of Environmental Health Sciences of the Department of Health, Education and Welfare, in collaboration with the National Environmental Research Center of the Environmental Protection Agency, organized a conference on the Biological Effects of Ingested Asbestos, which was held in Durham, North Carolina, November 18–20. 1973. Realizing that our biological knowledge was at best scanty, and that an understanding of the physical and chemical properties of asbestos fibers, as well as their pathological potential, was necessary to an analysis of the processes involved, the two agencies, in conjunction with Dr. I. J. Selikoff, Director, Environmental Sciences Laboratory, Mt. Sinai Medical School, New York, cast their net widely and invited geologists, crystallographers and chemists as well as the usual array of biomedical scientists to pool their knowledge and suggest ways of arriving at a better understanding of the problem. In the search for relevant information and ideas, particles other than asbestos were included in the considerations. It was emphasized that, while prepared talks would be welcome, considerable attention would be paid to the discussion and to the reactions provoked by evidence and ideas advanced from many different points of view.

Formal papers presented at the conference or subsequently submitted, commentaries, and extracts from some less formal presentations, are included in these proceedings, in an order paralleling the sequence of this report, which has been prepared from these submissions,

*3204 Arrowwood Drive, Raleigh, N.C. 27604.

supplemented by notes made at the time and transcripts of recordings. The interpretations and opinions expressed are, naturally, those of the reporter, and should not be attributed to the individual participants. This overview makes no pretence at summarizing all available knowledge, but restricts itself to the information presented at the conference, with only such additional material as is necessary to give continuity and to put the discussions in perspective, mainly for the interested reader who is not himself a specialist in the field.

As pointed out by Dr. D. P. Rall, the Director of NIEHS, there are six parts to any systematic toxicological inquiry: (1) chemical identification and analysis; (2) the fate of the material in the environment; (3) the route of exposure for man; (4) the modes of absorption, distribution, metabolism and excretion in the organism; (5) the biological activity of the material; and (6) its mechanism of action. While this order is not exactly followed, readers will find that all of these considerations have been covered in the proceedings. Somewhat to the organizers' surprise, more studies were in train and more information was available than had been anticipated. While the material is still far from making a well-integrated whole, there is promise of a better understanding of the significance of ingested asbestos in the not too distant future.

Biologically Significant Material

The obvious question to be asked concerns the distribution of asbestos in water and the risk that biologically significant quantities might be ingested. This question is, indeed, uppermost in the minds of many, but like so many obvious questions, it is far too simplistic to permit a categorical answer. To most people asbestos is an entity that should be capable of recognition and measurement; but to those studying the material it is far from being one specific substance. As will be immediately evident from many of the following papers, the term "asbestos" covers a wide range of similar but far from identical minerals. The great proportion of asbestos material is of the type called chrysotile, in which the molecular structure is such that the ultimate fibril, of which fibers and bundles of fibers are composed, takes the form of a minute tube. This material is of fairly uniform composition and can be identified fairly easily under the electron microscope. It approximates to the general idea of a single entity. Contrasted with this is the remaining portion of asbestos minerals, which have a different basic molecular structure and vary in detailed composition, going under the generic name of "amphiboles".

As will be seen in the paper by Bowes, the proportions of magnesium, iron, calcium and other elements in the amphiboles can vary over a three-dimensional spectrum. Specific names are given to certain combinations, such as crocidolite or blue asbestos, anthophyllite, amosite or the cummingtonite–grunerite series (the type mainly seen in the Duluth situation), tremolite–actinolite, and so on until we finally arrive at talc, which to the lay mind is not usually thought of in connection with asbestos. As might be expected, the borderlines between these various types are the result of geological vagaries and are somewhat indefinite. The papers by Rohl and Ross indicate, for example, the variation from anthophyllite through tremolite to talc in a New York State mine. At the other end of this confusing array one encounters the name serpentine, which denotes a very general class of minerals including chrysotile. It is clear that geologists and mineralogists can render valuable service in determining what class of material a given deposit is likely to yield and in monitoring the variations as they are encountered.

The essential quality that justifies the name asbestos being given to a particular member of this class is its fibrous character, the property of readily breaking up into fine fibers that can be worked and even spun as such. It is ironic that this very property which gives the material its commercial value also makes a meaningful measurement of its distribution as a contaminant or pollutant uncertain. If the biological activity depended simply upon the quantity of material taken into the body, then one would need only to measure its concentration by weight in the medium (air, water or food), as one does for many chemical agents. Unfortunately, this simple relationship does not hold for asbestos. Considerable attention has to be paid to the concentration of particles, to the size distribution of those particles, and even to the surface area presented by those particles, since the biological effect is believed to be linked to

these features rather than to the total mass. Because of its facility for fragmentation, a given quantity of asbestos can produce an extremely wide range of particle sizes and numbers. The awkward question immediately arises: "What is a particle?" or more precisely, "what constitutes a biologically effective particle?" Here is where the uncertainty starts.

Mixed up with this uncertainty is another question, "What is a fiber as opposed to a nonfibrous particle?" For some, a particle whose length is three times its mean breadth is a fiber; others demand as much as a ten to one ratio. The definition is more than academic, since some at least of the properties that make asbestos biologically active are believed to be associated with its fibrous shape. Then there is the question of fiber size. Some stand by the doctrine enunciated several years ago that only fibers whose length lies between 5 and 25 μm are significant, and the current OSHA standard for air-borne asbestos includes this limitation. (The OSHA method also simply counts all fibers, which is acceptable where the great preponderance is known to be asbestos, but not where identity is in doubt.) Many, however, are firmly convinced that it is the submicroscopic particle that can penetrate cells and cause damage, although they may be fairly rapidly degraded, and that these are the ones that should be measured. Since one large fiber can break up into a multitude of small fibers, a resolution of this disagreement is urgently necessary.

On top of all of this is the nagging thought put forward by some that perhaps it is not so much the fiber itself that matters, but rather what it may carry on its surface or combined with its molecules. Variability in the inherent content of metals such as Ca, Mg, and Fe has already been mentioned. To this must be added variability in materials such as Ni, Cr, Co, benzo-[a]-pyrene and other agents suspected of carcinogenicity, that can readily be adsorbed on the surface and thus be carried into tissues as the fiber penetrates.

If one is to make meaningful measurements of a biologically active substance in a medium such as water, it is highly desirable that the measurement be confined to that substance and not include similar but biologically nonsignificant material. From what has been said, however, it is clear that we are far from being able to do this with precision for asbestos. We are uncertain about too many aspects that affect its biological significance: the type of asbestos, the dimensions of the fiber, the exact mineral composition of the fiber, and the importance of materials that might be adsorbed on or incorporated into it.

We are, therefore, in the unfortunate position of having to measure the concentration of biologically significant asbestos in a medium such as water without being altogether sure of what should be measured. To cover various needs, the following information would be highly desirable: (1) identification and classification of the various types of asbestos fiber present in the material being ingested; (2) distribution of particle size; (3) the total concentration; (4) chemical analysis to determine any deviations from the basic molecular structure.

The extent to which such comprehensive information can be obtained in practice depends upon the quality, reliability, and coverage provided by the available techniques and also, most importantly, upon the feasibility of applying those techniques to a sufficient number of samples.

Techniques of Identification and Measurement

Several of the contributions, notably those by Langer, Maggiore, Rohl, Stewart, Eichen, and Heinrich, discussed the techniques of identification and measurement in detail. Not all, unfortunately, have been turned into formal presentations.

The initial step of filtering a known volume of the medium to be examined, or of an extract from solids, involves the selection of a pore size appropriate to the dimensions of the particle to be retained. Fibers introduce a degree of uncertainty. At the beginning of filtration some fibers whose diameter (but not length) is smaller than pore size may be streamlined and pass through the filter; at later stages, when material has accumulated on the filter, particles smaller than pore size may become entangled and retained. The behavior of a given filter needs to be ascertained by test with material of known dimensions.

The material retained usually includes a proportion of substances other than asbestos.

Organic material can be removed by careful ashing at a temperature lower than that which affects fiber composition. The residue is available for chemical analysis and microscopic examination. Techniques of transfer are mentioned in some of the papers.

The identification of fibers in tissues presents some additional problems. If the precise relations of fibers to tissue components are under review, then sections must be cut with all components in place. The hardness of the fiber sometimes makes this difficult, and material may be forced by the microtome knife into false positions. If one simply wants to find out what is in a tissue, then digestion with some mild agent such as sodium hypochlorite, which does not change the nature of the fibers, is desirable. Care must be taken, of course, to see that material adhering to the outside of the tissue is not included. Something of a compromise can be effected by cutting a section and then ashing it in place on the microscope mount so that the disposition of the particles can be seen. (See papers by Fondimare, Le Bouffant, Bignon).

Light microscopy, including phase contrast and polarized light, is adequate for the counting and measurement of fibers down 5 μm in length, and fairly good down to 1 μm. It permits the recognition of most asbestos fibers as opposed to other mineral particles. It is adequate, therefore, for those who wish simply a total count of fibers of length greater than 5 μm. It does not, however, permit the estimation of fibers of submicroscopic size, and is not altogether adequate for determining the type of asbestos fiber present. Light microscopy does have the advantage of presenting fairly large fields, so that fewer fields need to be counted for statistical satisfaction.

The transmission electron microscope (TEM), in which a beam of electrons is used instead of photons in the visible range, has a much greater degree of resolution and permits much smaller particles to be observed, down to 10 Å or better; the field observed is much smaller, however, so that many more fields must be counted to satisfy statistical needs. Unfortunately, however, the fiber is seen in only two dimensions, and while chrysotile fibers are usually to be recognized by the tubular structure of the fibrils, various types of amphibole fibers cannot be distinguished from each other. If, however, the electron beam is focussed on a point on the fiber (say, 0.5 μm in diameter) instead of covering a finite area of specimen, an electron diffraction pattern is obtained which may permit determination of the mineral group and partial differentiation of the types. If, however, the particles in a field are of mixed type, particularly if foreign inorganic matter is present, the desired signals become obscured in a high "noise" background. Very small and very large diameter fibers also present special problems. In this work, also, it is generally undesirable to ash the specimen for fear of changing its composition, and thus of interfering with identification of the fiber. Nicholson's paper describes the technique of direct transfer of filter material for avoidance of artifacts, and also methods for breaking up agglomerates.

In the scanning electron microscope (SEM), the electron beam is focussed to a point which is then swept across the designated area of the specimen. Transmitted or scattered electrons can be picked up by suitably placed detectors. The current is amplified by photomultipliers and sent to the controls of a TV screen synchronized with the sweep of the electron beam. An image of the specimen is thus obtained and a three-dimensional view of the fiber may be seen. This can help in identification, but the resolution is relatively poor, going down only to 200 Å under ideal circumstances. The instrument can also be used as an electron microprobe, with energy dispersive or wavelength dispersive detectors picking up the radiation generated by the impact of the electron beam on a selected spot of the specimen. The output of the detectors can be used to plot out the intensity of the radiation by energy level or by wavelength and thus yield information on the chemical composition of the point under examination. Unfortunately, the collection of such information at several points along a fiber takes a good deal of time and the specimen may be modified by the beam.

A combination of techniques is desirable, and various assemblies have been devised. All of the techniques, however, suffer from the amount of time that they demand in preparation, performance, and interpretation, particularly when applied to material of unknown composition or provenance. The collection of samples is a

relatively easy matter, but the elicitation and compilation of information derivable from those samples are very time-consuming and expensive. It is not the kind of activity that a research team likes to undertake, but neither is it the kind of activity for which routine facilities and operators are generally available.

Automation has been proposed for the compilation of information without undue involvement of the operator's time. For automation to be successful, however, the number of characteristics to be recorded must be great enough to include the essential points, but small enough for the examiner to be able to recognize diagnostic patterns in the emergent information. Too much information can inhibit comprehension; too little may miss the mark. The items to be recorded, therefore, need to be carefully selected and standardized. Eugene White described a combination of SEM and x-ray spectrographic modes, computer controlled to scan very rapidly when no particles are in view, and more slowly when they are. Automated characterization is much easier when the material is of uniform composition than when it is mixed or contains much foreign matter.

Methods of detection and measurement have been so pushed to the limits of their sensitivity, that a tenfold variation can be expected in replicate analysis, and considerable background "noise" is experienced. Ancillary techniques, listed by Langer, include infrared spectroscopy, differential thermal analysis, emission spectroscopy, atomic absorption spectroscopy, and wet chemical analysis. The latter is applicable only to bulk material. Chrysolite may be separated from amphiboles, but not amphiboles from each other, by electrophoresis. These methods, also, are time-consuming and have only limited applications.

Distribution in Potential Ingesta

The paper by Speil presents what might be called the background picture of asbestos fibers in natural water supplies. Using a mass technique, he finds the percentage of chrysotile fiber in the filter residue to be 0.001–0.5% of the total matter on a weight basis. The sample is ashed at 800°F to remove most of the organic matter, and the rubbed-out residue is examined by TEM. The length and diameter of each chrysotile fiber are measured in an appropriate number of fields, and the mass of the chrysotile in the sample is calculated. In the water supply of ten cities the content was found to vary between 0.005 and 6.0 μg/gal. All of the fibers were of submicroscopic size; they were not visible with the light microscope.

Comparison of water from a river running through serpentine rocks with water from one not so exposed somewhat surprisingly showed no significant difference. It should be noted, however, that there was great variability in the content of both rivers from point to point and from time to time. Replicate samples also showed a wide variability even when analysed by the same operator. A very large number of samples would be necessary to show inherent differences in the face of such variability.

If one assumes that a person drinks 2 l./day over a 70-year lifetime, the total fiber intake from such water would be somewhere between 0.05 and 0.3 g. While this sounds like a trifling amount, each microgram would represent 1 million fibers measuring 1.5 × 0.15 μm. Speil, Langer, Pontefract, and others have found asbestos fibers in such diverse media as beer, wine, and water from the polar icecap. The biological significance of such background concentrations will be discussed later.

Nicholson reported that he found 20 to as high as 70 million fibers of amphibole minerals per liter in the drinking water system of the city of Duluth, as compared with the 6 million particles of chrysotile per gallon (1.5×10^6/l.) reported as background by Speil. The majority of the fibers found by Nicholson were less than 1 μm in length, and the most probable diameter was 0.2 μm. All of the fibers had a ratio of length to diameter of 3:1 or more. Roughly 50% of the material was in the cummingtonite–grunerite series. Nicholson did not find detectable amphibole asbestos in the drinking water of New York City or in that of Grand Marais, Minnesota, which is up-current from the discharge into Lake Superior. Nicholson warned against facile comparison of fiber counts made by different methods, particularly where chrysotile is the object of search, as many methods break up the fibers.

The question is frequently raised as to

whether the use of asbestos cement pipes for the carriage of drinking water supplies, which has been increasingly the practice over the last 25 years, would add significant amounts of asbestos to the water. The general opinion expressed was that the amount would be very small and constitute no more than a negligible addition to the general background. The crocidolite used in asbestos–cement pipes, which does not occur in nature in the U.S. aquifers, could be used as a marker to test this conclusion (Selikoff). Webster's paper contains the following relevant paragraphs, supported by photographs:

> "In pipes carrying hard water (with a positive saturation index) the asbestos–cement pipe shows little change even after many years. Even with a negative saturation index as in soft water there is little damage to the asbestos–cement pipe and organic material is deposited on the inner surface. With high saturation indices there is a well marked deposition of calcium salts lining the inside of the pipe as shown by a pipe which was carrying brine near the Koegas mine or salt at one of the asbestos–cement factories. It is therefore difficult to envisage contamination of drinking water which is hard and reticulated through asbestos–cement pipes."
>
> Sewage, however, is acid and will corrode the asbestos–cement pipes due to the release of carbon dioxide and the formation of sulfuric acid through baterial action. Now that such water is being treated and used for industrial purposes, there are many investigations underway to determine whether the water is fit for human consumption. It is unlikely that asbestos fibers will be found, but should such be the finding the introduction of a flocculating filtration system will prevent fiber contamination."

As indicated in the paper by Eisenberg, talc is commonly used in drugs as an excipient and as a dusting powder. It may be added to foods as an anisticking agent. From what was said earlier, it is not surprising to find that the talc is usually contaminated to a degree by its fellow amphibole, tremolite (Ross). As sources of relatively pure talc rock are used up, a greater degree of contamination is possible. The number of fibers per miligram corresponding to 0.1% contamination varies from 12 for the longer-fibered chrysotile to 125 for the shorter tremolite. Unfortunately, most reliance has been placed on light microscopy for the detection and measurement of asbestos fibers in food and drugs. Data more easily comparable with that being obtained for water will need the adoption of submicroscopic methods along the lines already mentioned. (The rules proposed by the Food and Drug Administration have already been set out in the Federal Register Vol. 38, No. 188, pp. 27076–27081, for September 28, 1973).

Asbestos filters are a potential source of contamination of liquids such as beer, and of parenteral biological drugs. The use of such filters is being controlled. While there has undoubtedly been exposure to the ingestion of asbestos particles from these sources in the past, the extent is not well defined. The instance quoted by Speil of unfiltered beer, made from spring water in serpentine rock, having the highest count indicates that perhaps the exposure was not greatly in excess of the background level.

Passage Through Gastrointestinal Wall

As pointed out by Webster, Brown, Holt, and Pontefract, and by others in the course of discussion, there is no doubt of the ease with which asbestos can move about in tissues, if not inside cells at least between them, so that passage through the gut wall would not be unexpected. There has been a fairly wide assumption, as pointed out by Zaidi, that one of the routes by which inhaled fibers can reach abdominal tissues is by passage up the tracheobronchial "escalator", followed by swallowing and passage through the intestinal wall. Laskin estimated that 25–50% of inhaled fibers are eventually cleared from the respiratory tract via the escalator and subsequently swallowed. There has been some doubt, however, whether there is direct evidence to support this reasonable assumption.

Davis and his colleagues point out that the epithelial cells of the intestinal wall are very tightly arranged, and that the penetration of the membrane may not be easy. Volkheimer's paper provides a detailed and striking demonstration of the penetration of the wall by various dietary particles. His illustrations relate mainly to rounded particles such as starch grains, but in his text he states that silicate crystals, powdered asbestos and diatomaceous particles behaved in similar fashion. Westlake, reporting

on the conduct of feeding studies in rats with 6% chrysotile added to the diet, found small chrysotile particles in the mucus of the goblet cells, of the colon, in the cytoplasm of the epithelial cells, and down into the smooth muscle of the wall. A possibility that the microtome knife had pushed fibers from the gut lumen into the cells was fairly well eliminated. In Volkheimer's experiments only large particles 20–70 μm in diameter were observed. As these appear in the blood stream in 1 min and reach their peak in 10 min, this part of the process is too fast to be attributed to passive processes. These persorption experiments were mainly carried out in fasting animals, and even then Volkheimer estimates that only 1 particle in 10,000 gets through the wall. Intestinal motility, caffeine, and smoking increase the process. If such large particles can penetrate the wall, and as red cells cross all manner of membranesm as pointed out by Brown, surely small fibers can. Pontefract reports that he found fairly numerous asbestos fibers in the blood of rats after injection by long needle through the wall into the lumen. The possibility that the wall had been contaminated from the needle was raised, but the counts obtained would seem greater than could have been occasioned by such contamination. In the course of discussion, Pontefract reported easy penetration of the wall by large glass fibers introduced to the stomach by intubation. After intubation of tritiated asbestos, radioactivity quickly appeared in the liver, kidney, and spleen, but this could have been leached out of the fibers while they remained in the gut.

Webster's paper reports the failure of asbestos-contaminated food and water fed to baboons for 5 years to produce peritoneal or gastrointestinal tumors. It should be noted, however, that if baboons react like man, 5 years is too short a time for tumors to develop. He indicates, on the other hand, that crocidolite fed by stomach tube to baboons resulted in the presence of iron particles in the macrophages of the duodenal and ileal mucosa. Only an occasional asbestos needle was found by electron microscopy in the ashed tissue of the stomach. In at least the early stages of a chronic feeding experiment by Davis, no fibers have been found in the gut wall by electron microscopy.

From what was presented and from the subsequent discussion, there seems to be little doubt that asbestos fibers can penetrate into and pass through the gastrointestinal wall. The question really is whether they actually do so under the conditions normally prevailing in the human alimentary canal, or at least in sufficient quantities to constitute a serious threat of carcinogenesis in the bowel wall or of mesothelioma in the peritoneum.

Fibers ingested in the normal way may remain dispersed in the bowel contents most of the time, coming into contact with the wall only occasionally, and even then tending to be dragged along with the contents. Angulations in the bowel may, however, hold material up for days. The common sites of carcinoma—cecum, sigmoid colon, and rectum—provide such opportunities. The situation in the bowel, where the contents are moved more or less steadily along its length, is quite different from that in the lung. The concentration of fibers in inspired air may be much higher than that in ingesta, the fibers are dispersed in air free from interfering matter, there is every opportunity for every fiber to come into contact with the airway walls, and the deadend situation provided by the terminal bronchioles and air sacs serves to retain those fibers that are deposited, unless they can get on the lowest part of the tracheobronchial escalator. In both the gastrointestinal tract and the airways of the lung, normal movements of the parts would tend to increase contact and penetration of the epithelium and subsequent migration. In the bowel, as in the respiratory tract, mucus serves as a protective coat. Deficiencies in the coat may provide sites of easy penetration.

The number of fibers that pass through the bowel wall would seem, in the light of the above considerations, to be small. They would also tend to be of microscopic size. In view of this and of the limitations of the usual techniques for fiber detection and identification (and particularly for fibers in tissues), the chances of actually catching fibers in passage would seem to be low. Failure to find them after feeding experiments can hardly be regarded as strong evidence against their passage; we are talking about concentrations of 10^{-12} g of material, less than that sought in trace metal analysis. Failure

of experimental animals to develop bowel cancer or mesothelioma after oral feeding of asbestos, likewise, cannot be taken as indications of safety for man, so great is the variability of histological anatomy and susceptibility between species, and so long the time interval required for the appearance of tumors after initial exposure. Short-lived animals hardly provide a fair test. Failure to find asbestos fibers in tumors of the abdomen also provides no more than weak negative evidence; carcinogens may disappear long before the effect of their operation in the production of a clone of malignant cell is evident. When we remember, also, the low exposures to inhaled asbestos that can be followed by mesothelioma in man, the significance of even a few fibers passing across the bowel wall may be important. How great the risk is only time will tell; but until then it cannot be ignored. An early realistic estimate of risk is highly desirable. If it is small, then the expenditure of considerable resources to effect further reduction might be questionable. In the meantime we can only speculate, and one man's judgement of an acceptable risk notoriously differs from another's.

Tissue Reactions

The question of whether and to what extent asbestos fibers pass into or through the gastrointestinal wall is of importance only in terms of the tissue reactions that are likely to be set up by the fibers after passage. There is already a very extensive literature on tissue reactions to asbestos, but very little of it relates specifically to the bowel. Most of the work has been done in connection with the response of lung tissue to inhaled fibers, or to material injected into the trachea. Studies have also been made of tissue reactions following injection into the pleural and peritoneal cavities, and of applications to the pleural surface.

Several excellent accounts of such reactions were presented at the conference and appear as papers in this issue. Since there is little that is specific to the gastrointestinal situation, a brief summary of the highlights will suffice to put our problem in perspective.

Suzuki provides an excellent set of electron micrographs illustrating the process of phagocytosis of asbestos fibers. These support the idea that the plasma membrane invaginates with the fiber, and continues to surround and to separate it from the cytoplasm itself. Presumably it is this vacuole or phagosome that serves as a lysosome or into which lysozymes are secreted. It was clear from the discussion that some believe that the fiber can actually pierce the cell membrane. This would be an academic point, perhaps, did it not affect concepts of the mechanical action of the needlelike fibers. Davis, for one, considers phagosome formation a further argument against mechanical penetration of the bowel wall. In any case, iron–containing particles are found in the phagocyte in response, presumably, to the presence of a fiber, and cytoxic effects are also evident in the cell.

The cytotoxicity of chrysotile fibers in particular is well brought out by Schnitzer, Hayashi, Pott, and Harington. Damage to macrophages as a result of such toxicity is seen as an essential step in the initiation of fibrogenesis. The extent to which surface reactions involved in cytotoxicity can be varied is also indicated by the same authors.

With regard to the fibrogenic mechanism, we can hardly do better tnan. cite excerpts from the very clear account given by Harington:

> "The death of the macrophage is a prerequisite to the subsequent fibrogenesis, although the biochemical steps between these two processes have not yet been worked out. All forms of asbestos fibers can stimulate the production of collagen in experimental animals." . . .
> "There is now evidence that the state of the cell or tissue culture during experiments may be a deciding factor as to whether stimulation or inhibition of collagen production takes place." . . .
> "There are three possibilities to consider. (a) Dead or dying macrophages under appropriate conditions release a factor which stimulates fibroblasts to produce collagen. . . .(b) Dead or dying macrophages. . .are attended by a rapidly increasing number of fibroblasts making constant amounts of collagen. . .(c) A combination of both. . ."
> "The synthesis and laying down of collagen *in vitro* is a complex process which can be affected by many variables including the composition of the medium, the rate of growth of the cells, the proportion of collagen synthesized that is bound within the cell mat and the breakdown of collagen itself.". . . .

> "Fiber diameter has received considerable attention and seems to play an important part in deciding how deeply penetration into the lung can occur. . .and whether some types of fiber may induce mesothelioma in man or not. . ."

Other papers relating to the process of fibrogenesis are those by Chvapil, Heppleston, and Holt. The importance of interactive synergism was pointed out by Holt, who found fibrosis in routine but not in SPF experimental animals.

The carcinogenic effect of asbestos fibers is, of course, very relevant to the concerns of the conference. Two types and locations need to be considered: epithelioma in the wall of the gastrointestinal tract and mesothelioma in the peritoneum. An increasing incidence of colonic and rectal cancer, concomitant with a decreasing incidence of gastric cancer, was noted in the general U.S. population. Statistics compiled by the American Cancer Society indicate that there were 43,000 deaths in the U.S. in 1971 from colonic and rectal cancers. Selikoff predicted that the incidence of cancer of the lung, of the breast, and of the colon would be about the same in the U.S.

Speculation naturally developed on the extent to which ingested asbestos, even at background levels, might play a part in the increase in colonic cancer. The incidence in asbestos workers is three or four times the expected rate, but the geographic incidence in the general population does not correspond to apparent exposure. The possible routes by which inhaled fibers might reach the abdomen were reviewed. The escalator route followed by swallowing of the cleared fibers has been mentioned, but fibers could very well pass more or less directly from lung tissue to abdominal sites. Techniques of bowel examination were presented and the presence of some asbestos fibers in the colonic wall of experimental animals was mentioned. But beyond this, no substantial evidence was provided for the association of the increase of gastrointestinal cancer with ingestion of asbestos. Very little was said either about the production of mesothelioma beyond its appearance in certain experimental animals after intraperitoneal injection.

Theories of carcinogenesis in general and possible mechanisms by which asbestos in particular might act naturally provided much discussion. A demonstration by Amacher of increased DNA production in rats after asbestos ingestion raised the possibility that the fibers may be provoking increased cell multiplication, and that this may favor the establishment of a clone of cells mutated by some other carcinogen. The exact metallic components of the fiber, bound or adsorbed, were suggested as etiological factors. The situation is very confusing. Vostal reported that, whereas Mn, Zr, and Ti occur more prominently in amosite, Ni and Cr are in higher concentrations in chrysotile.

The idea that any fiber is potentially carcinogenic had its adherents. Stewart emphasized that great species and even strain variability in carcinogenic susceptibility and appealed for the use of a wide range of animal types in testing procedures. The importance of considering critical combinations of multiple factros was stressed by Epstein. The importance of synergism is now widely accepted, but the practical problem of dealing with multiple factors remains. It is very difficult to find populations for epidemiological study free from the confounding factors of smoking, for example.

Research Directions

It is quite evident from the foregoing review that the conference, as fully expected, raised more questions than it answered. Its purpose was to review what was known and to suggest how the uncertain state of our knowledge about the effects of ingested asbestos might be remedied. It served well in exposing the uncertainties, in alerting all concerned to deficiencies in current investigative techniques, and in engaging the interest of a wide variety of investigators. It transpired that there was much more activity in the field than the convenors had realized, and many participants learned of work that was previously unknown to them. As revealed by the discussion, among the more urgent items that need to be resolved are: more precise methods of detecting, identifying, measuring and counting asbestos fibers in water and food; standardization of information to be obtained on asbestos fibers in environmental media, and automation of the data gathering

process; sytematic determination of the distribution of asbestos in water and food; determination of the extent to which fibers are modified during long immersion in water; conduct of epidemiological surveys in which environmental information on asbestos is gathered in conjunction with data on body burdens, functional disturbances and disease states; clarification of the extent to which inhaled asbestos is subsequently swallowed and added to the gastrointestinal burden; resolution of the relative importance of long and short fibers in the induction of biological responses; intensive study of the effects of food, gastrointestinal motility, and physiological states on the opportunities for fiber contact with and passage into the bowel wall; systematic measurements of the dose/response relationships in the production of epithelioma and mesothelioma under natural gastrointestinal conditions; continued investigation of the mechanisms by which fibrosis and neoplastic changes are produced by asbestos fibers, having regard to fiber type and composition.

In these investigations it was emphasized that greater attention should be given to the following: standardization of techniques and protocols; cooperative studies by different groups of investigators; selection of experimental animals which are histologically and immunologically suitable for the response being studied; coordination of environmental data with biological effects in the conduct of epidemiological and surveillance studies.

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